FINAL REPORT

MARCH 31, 1991

DSS CONTRACT #23440-9-9304/01-SQ

Entitled: The Development of Bacterial Biofilms for the Selective Removal of Metals from Mining Effluents

Respectfully submitted

J.W. Costerton
President

Microbios Ltd.
3823 Brooklyn Cres. N.W.
Calgary, Alberta
T2L 1G9

(403) 220-7301

Scientific Authority

Dr. R.G.L. McCready
Biotechnology
CANMET - EMR

(613)992-1596

17pp

MICROMEDIA

EXECUTIVE SUMMARY

Microbiologists have known for several decades that microbial cells often have a remarkable ability to bind metals. This binding capacity, with additional binding by microbial exopolysaccharides, is currently used very successfully in the commercial recovery of copper, uranium, and selenium from mine waters and mining effluents.

Because Falconbridge Nickel wishes both to recover valuable metals from its mining effluents and to improve its environmental interface by releasing waters with acceptable metal contents, we have undertaken a study of the microbial binding of metals that are present in Falconbridge effluents by bacteria that are also present in these effluents. Using bacteria that are naturally present in Falconbridge effluents, we have discovered strains that bind Ni, Co, and Cu very avidly ($\pm$ 3 gm/gm of bacterial biofilm). Careful strain selection has identified organisms that bind large amounts of Ni without binding significant amounts of Fe. The use of dead biofilms of selected strains enhances the binding of Ni and Co and further reduces the binding of Fe. Manipulation of the pH level at which dead bacterial biofilms are exposed to aqueous solutions of metals resembling those found in Falconbridge effluents further enhances the binding of metals, especially Cu.

These data indicate that microbial binding could be very successful in the commercial-scale recovery of Ni, Co and Cu from Falconbridge effluents. This recovery could be effected by the manipulation of effluent stream conditions (eg. nutrient stimulation, provision of suitable solid substrata, etc.) to stimulate the development of mixed biofilms of natural microbial populations that would bind large amounts of valuable metals. This slimey biofilm material could be harvested and degraded (0.5% bleach) to release bound metals for commercial recovery. Alternatively, biofilms of metal-binding bacterial strains could be produced, killed, and used to produce pH conditioned pellets that would be used as ion exchange materials for the specific commercial recovery of valuable metals (Ni, Co, Cu). Both applications of microbial metal binding would sharply reduce the metal content of Falconbridge effluents at their environmental interfaces.

RESUME EXECUTIF

Les microbiologistes savent depuis des décennies que les microbes peuvent lier certains métaux. Cette capacité, renforcée par les exopolysaccharides des microbes, est actuellement utilisée avec grand succès dans la séparation commerciale du cuivre, de l'uranium et du selenium des eaux minières.

Falkonbridge Nickel désire séparer les métaux riches de ses effluents miniers et améliorer leur impact environnemental en éliminant des eaux ayant un contenu en métaux acceptable. En conséquence nous avons entrepris une étude de l'interaction des microbes avec les métaux dans les effluents de Falkonbridge. Nous avons découvert des lignées bactériennes qui lient avidement le Ni, le Co et le Cu ($\pm$ 3 gm/gm de biofilm bactérien). Une sélection précise de plusieurs lignées a permis l'identification d'organismes qui lient le Ni en grandes quantités sans lier le Fe. En manipulant le pH des solutions aqueuses contenant les biofilms bactériens morts, dans des conditions similaires à celles rencountrées à Falkonbridge, nous avons réussi à améliorer la capacité bactérienne de lier les métaux, le Cu en particulier.

Nos résultats suggèrent que cette faculté bactérienne pourrait être utilisée avec succès lors de la production commerciale de Ni, de Co, et de Cu à Falkonbridge. Ceci pourrait être réalisé par la manipulation des effluents, stimulation par des nutrients ou addition de substrats solides appropriés par exemple. Ces modifications permettraient de stimuler le développement de biofilms bactériens mixtes par les populations endogènes et ainsi conduiraient à une production métallière améliorée. Ces biofilms pourraient être recueillis et dégradés (par 0.5 % d'eau de Javel) afin d'en séparer les métaux riches. D'autre part, des lignées de bactéries produisant des biofilms liant les métaux pourraient être établies. Ces microbes seraient alors tués et utilisés pour la production d'éléments conditionnés par le pH; de là, ces éléments seraient utilisés comme matériaux d'échanges ioniques pour la production commerciale de métaux riches (Ni, Co, Cu). Ces deux applications des capacités bactériennes de lier des métaux pourraient réduire le contenu en métaux des effluents de Falkonbridge.

BACKGROUND

Many microbiologists have noted the very avid tendency of bacterial cells and their exopolymers to trap heavy metal ions. Fungal biomass is presently used by CANMET Biotechnology to trap uranium on a commercial scale and selenium is trapped and recovered by microbial biofilms in another CANMET operation. Biomaterials, which have an almost infinite chemical variability, may be very selective in their metal trapping specificity. Most metal recovery processes begin with an inventory of the thousands of available organisms and an assessment of how their specific metal trapping characteristics coincide with the objectives of the industry concerned.

Falconbridge Ltd. have experienced environmental problems because effluents from some of their remote sites have been found to contain large amounts of nickel. We proposed to address this problem by the use of biofilms of natural aquatic bacteria to trap this metal, along with other metals (eg. Co, Cu) of environmental importance, and thus to remove a hazard to the environment. Then, at the urging of Dr. McCready (Scientific Authority), we added the objective of recovering commercially important quantities of nickel during this solution of an environmental problem.

Initially, as was originally planned, we sought simply to establish that bacteria existed where sessile biofilms would trap valuable metals (Ni, Co, Cu). Instead of using bacteria from our environmental culture collection, we used several isolates from our acid coal mine drainage stream in Canmore, Alberta that had some characteristics in common with the Falconbridge sites. The data from this study (Milestone Report #1, July 30, 1990) clearly showed that some isolates trapped Ni, Co and Cu very avidly and somewhat selectively.

We then expanded Objective #2, which had originally committed us to test the ability of organisms from Objective #1 to trap metals (Ni, Co, Cu) from Falconbridge effluents, by travelling to Sudbury and isolating nine new aquatic organisms from three sites at Falconbridge. We analyzed a representative Falconbridge effluent and produced artificial Falconbridge water (AFW) which resembles this effluent in metal content. We replaced our older Robbins Device method with a method in which selected bacteria formed biofilms on glass beads of known surface area and were then exposed to AFW to determine their metal trapping capabilities.

Because we had resolved to aim for both metal removal and for commercial metal recovery, we then modified Objective #3. We concluded that we needed a bacterial material that binds Ni but does not bind Fe, which complicates the smelting process, and that we should develop a biomaterial that could be used repeatedly for metal trapping and release on a commercial scale. So, instead of developing biofilm reactors for metal removal alone, we resolved to develop a bacterial process material for the removal and commercial

recovery of Ni, Co, and Cu. We selected two isolates from the Falconbridge system and we determined that these bacteria could bind valuable metals, specifically, when they grew in living biofilms on glass beads. Dead biofilms of these organisms often showed more avid and more specific metal binding characteristics that are of potential interest. Further, adjustment of the pH of the AFW often radically improved the already favorable metal binding characteristics. In essence, we modified our original plan to control the release of metals into the environment in order to develop a promising biotechnological system that will both control environmental release and recover valuable metals.

ORIGINAL OBJECTIVES

1. To test biofilms formed by a large number of pure aquatic microbial isolates for their ability to trap Ni, Co, Cu, and precious metals (Aq, Au, Pl) from a standard mixture of metals.

2. To test the ability of biofilms of the most successful isolates from Objective #1, which trap Ni or Co but do not trap Fe, to trap these metals out of mine effluents supplied by Falconbridge.

3. To examine a mine effluent system chosen by Falconbridge in order to determine whether the best trapping strains are naturally present in the effluent ecosystem, and to devise environmental parameters to encourage their predominance in natural biofilms.

Expanded Objective:

4. To develop a microbial metal trapping system that will remove valuable metals, selectively, from Falconbridge effluents and will fascilitate the commercial recovery of these metals.

PROGRESS TOWARDS OBJECTIVES

Objective #1

We isolated 22 strains of bacteria from acid coal mine drainage water (Canmore, Alberta) and discarded 5 strains that bound macroscopically discernible amounts of Fe. These pure cultures of bacteria were induced to form biofilms on Robbins Device discs and after 1-8 days, these colonized discs were transferred to Robbins Devices in which they were exposed, for 72 hours, to a solution containing Ni, Co, and Cu. Table 1 details metal binding by the 10 most successful bacterial isolates.

Table 1

Metal Binding by Bacterial Isolates from Acid Coal Mine Drainage

Isolate	Ni*	Co	Cu
2	220	40	20
3	80	120	300
6	120	80	60
8	250	100	70
9	210	110	90
10	480	50	80
13	40	90	130
14	100	70	310
18	380	80	760
20	20	50	30

The Ni Co Cu solution contained 4 µg/ml of Ni
1 µg/ml of Co
1 µg/ml of Cu

*Values are in $\mu g/cm^2$ of biofilm-covered surface area after 72 hours of exposure

---

We conclude that many naturally occurring aquatic bacteria have the ability selectively to bind specific heavy metals.

Objective #2

We retained three of the Canmore isolates because of their favorable Ni/Fe binding ratios, and we travelled to the Falconbridge sites in Sudbury in early September 1990 to install glass slide holders. These glass slides were retrieved in late September and nine strains of predominant biofilm bacteria were isolated from three sites in the Falconbridge operation. We developed an artificial Falconbridge water (AFW) and glass beads that had been colonized by each of the three Canmore and nine Falconbridge isolates were exposed for 72 hours to AFW containing 0.4 µg/ml of Fe and 0.1 µg/ml of Ni, Co, and Cu. Six of these isolates bound impressive amounts of Ni, Co, and Cu (Table 2).

Table 2

Metal Binding by Isolates from Canmore and from Falconbridge

Isolate	Ni*	Co	Cu
C 10	5.6	0.7	1.1
F 1	3.2	2.8	3.2
F 2	4.5	1.1	2.8
F 4	7.2	1.2	3.0
F 7	8.0	2.0	3.2
F 8	6.7	0.9	1.6

*Values are in μg/bead with the beads having a surface area of approximately 0.18 cm^2

We conclude that aquatic bacteria exist in Falconbridge effluents that have the ability to concentrate large amounts of Ni, Co and Cu into living biofilms.

Objective #3

This objective was largely superceded by the new expanded Objective #4 but it is clear from the data in Objective #2 that nutrient stimulation and the provision of additional surface area for biofilm formation would produce microbial biofilms that would trap most of the Ni, Co, and Cu in Falconbridge effluents, preventing the release of these metals into the environment.

Objective #4

Based on the data generated under Objective #2, two isolates (Falconbridge 2 and 8) were selected for further study. Isolates F2 and F8 were identified as Bacillus cereus and Serratia liquefaciens, respectively.

The general experimental protocol employed was as follows:

> The isolates were grown for 7 days in 1/10 BHI (Brain Heart Infusion) medium at pH 4 or pH 7 and induced to form biofilms on 3mm glass beads having a surface area of approximately 0.18 cm^2. Prior to metals exposure, the beads were divided into two groups in order to determine uptake by live and dead (ethylene-oxide gas sterilized) biofilms. After exposure to artificial Falconbridge water (AFW) containing 0.4 μg/ml of Fe

and 0.1 μg/ml of Ni, Co, and Cu for 30 hours, the beads were rinsed to remove unbound metals. The beads were then immersed in 10% HNO_3 and the resulting acid solution was analyzed for metal content using atomic absorption spectroscopy.

Trial 1: Both isolates were grown at pH 4 and pH 7, and the resultant live and dead biofilms were exposed to the following treatments:

a) control - no metals added. These values were subtracted from the levels measured in the remaining treatments.
b) exposed to AFW at pH 7.0
c) exposed to AFW at pH 7.0 and then acidified to pH 4.0
d) exposed to AFW at pH 7.0, acidified to pH 4.0, and then replaced with AFW at pH 7.0
e) exposed to AFW at pH 4.0

The metal binding data obtained in Trial #1 are presented in Tables 3 to 6. These data show that the metal binding properties of a particular microbial biofilm are profoundly altered by the viability of the biofilm and by the pH (ionization state) at which the metals are presented.

The following conclusions are suggested by these data:

1. Dead biofilms of F2 and F8 generally bind less Fe than live biofilms.

2. Significant Ni uptake was found only in the isolate 8 biofilm when grown at pH 4. The dead F8-pH4 biofilm exhibited greater Ni binding capability than the live biofilm. Exposure to AFW at various pH's did not enhance Ni uptake.

3. Isolate F8 biofilms grown at pH4 or pH7 were not capable of binding Co when alive or dead. Cobalt was bound only by dead F2 biofilms regardless of the pH at which the biofilm had been grown, but only in very small amounts.

4. As with the Ni uptake, significant Cu uptake was found only in the isolate 8 biofilm grown at pH 4. Copper uptake was enhanced dramatically in both the live and dead F8-pH4 biofilms when the biofilms were exposed to AFW at varying pH's (treatment "d").

Trial 2: Trial 2 was undertaken to further examine the pH effects noted in Trial 1. Isolates F2 and F8 were again grown at pH 7 or pH 4 to create biofilm-coated glass beads. To minimize Fe uptake, only dead biofilms were exposed to the treatments illustrated in Figures 1 and 2. The data are presented in Tables 3 to 6, and generally confirm the trends noted in Trial 1.

Table 3

Fe Uptake (g Fe/g Dry wt. Biomass)

Trial 1			Isolate 8	Isolate 2
pH7	Alive	b	1.2×10^{-4}	n.d.
		c	n.d.	n.d.
		d	2.0×10^{-4}	4.3×10^{-4}
		e	1.6×10^{-4}	8.5×10^{-4}
	Dead	b	1.4×10^{-4}	n.d.
		c	n.d.	n.d.
		d	n.d.	n.d.
		e	9.4×10^{-5}	2.0×10^{-4}
pH4	Alive	b	7.6	6.1×10^{-4}
		c	5.6	1.1×10^{-2}
		d	15.0	1.5×10^{-2}
		e	12.4	1.3×10^{-2}
	Dead	b	4.2	n.d.
		c	n.d.	8.7×10^{-3}
		d	1.6	n.d.
		e	n.d.	9.5×10^{-3}
Trial 2				
pH7	Dead	A	n.d.	6.7×10^{-5}
		B	n.d.	9.6×10^{-4}
		C	n.d.	2.3×10^{-4}
		D	n.d.	n.d.
		E	n.d.	n.d.
pH4	Dead	A	0.73	n.d.
		B	n.d.	n.d.
		C	n.d.	n.d.
		D	3.8	n.d.
		E	5.6	n.d.

n.d. = not detectable

Table 4

Ni Uptake (g Ni/g Dry wt. Biomass)

Trial 1			Isolate 8	Isolate 2
pH7	Alive	b	9.8×10^{-5}	n.d.
		c	1.2×10^{-5}	n.d.
		d	1.1×10^{-4}	n.d.
		e	4.5×10^{-5}	n.d.
pH7	Dead	b	n.d.	n.d.
		c	2.4×10^{-5}	9.8×10^{-5}
		d	1.4×10^{-4}	2.4×10^{-5}
		e	3.7×10^{-5}	1.3×10^{-4}
pH4	Alive	b	1.2	2.9×10^{-4}
		c	1.3	1.5×10^{-3}
		d	0.73	2.3×10^{-3}
		e	0.98	2.4×10^{-4}
pH4	Dead	b	2.4	9.8×10^{-4}
		c	n.d.	1.9×10^{-3}
		d	2.7	1.6×10^{-3}
		e	2.7	1.5×10^{-3}
Trial 2				
pH7	Dead	A	1.6×10^{-5}	n.d.
		B	6.1×10^{-5}	n.d.
		C	5.7×10^{-5}	n.d.
		D	9.0×10^{-5}	n.d.
		E	n.d.	n.d.
pH4	Dead	A	3.6	n.d.
		B	2.4	7.3×10^{-4}
		C	1.7	4.9×10^{-4}
		D	0.98	1.6×10^{-4}
		E	1.96	n.d.

n.d. = not detectable

Table 5

Co Uptake (g Co/g Dry wt. Biomass)

Trial 1			Isolate 8	Isolate 2
pH7	Alive	b	n.d.	n.d.
		c	n.d.	n.d.
		d	n.d.	n.d.
		e	n.d.	n.d.
	Dead	b	n.d.	3.7×10^{-5}
		c	n.d.	5.5×10^{-5}
		d	n.d.	1.2×10^{-4}
		e	n.d.	1.0×10^{-4}
pH4	Alive	b	n.d.	n.d.
		c	n.d.	n.d.
		d	n.d.	n.d.
		e	n.d.	n.d.
	Dead	b	n.d.	n.d.
		c	n.d.	2.4×10^{-4}
		d	n.d.	4.1×10^{-5}
		e	n.d.	1.6×10^{-4}

Trial 2				
pH7	Dead	A	n.d.	7.3×10^{-5}
		B	n.d.	1.8×10^{-5}
		C	n.d.	4.3×10^{-5}
		D	n.d.	5.5×10^{-5}
		E	n.d.	7.3×10^{-5}
pH4	Dead	A	n.d.	2.9×10^{-4}
		B	n.d.	8.1×10^{-5}
		C	n.d.	2.0×10^{-4}
		D	n.d.	n.d.
		E	n.d.	n.d.

n.d. = not detectable

Table 6

Cu Uptake (g Cu/g Dry wt. Biomass)

Trial 1			Isolate 8	Isolate 2
pH7	Alive	b	2.4×10^{-5}	6.1×10^{-6}
		c	6.5×10^{-5}	6.1×10^{-6}
		d	2.2×10^{-4}	4.3×10^{-5}
		e	1.2×10^{-4}	1.8×10^{-5}
	Dead	b	3.3×10^{-5}	1.2×10^{-5}
		c	4.1×10^{-5}	3.1×10^{-4}
		d	1.6×10^{-4}	3.7×10^{-4}
		e	6.1×10^{-5}	1.2×10^{-5}
pH4	Alive	b	0.36	2.0×10^{-4}
		c	1.1	2.4×10^{-4}
		d	3.6	2.9×10^{-4}
		e	0.98	2.0×10^{-4}
	Dead	b	0.98	1.6×10^{-4}
		c	0.61	9.0×10^{-4}
		d	2.5	8.2×10^{-5}
		e	1.3	9.0×10^{-4}
Trial 2				
pH7	Dead	A	2.9×10^{-5}	4.3×10^{-5}
		B	8.1×10^{-6}	7.3×10^{-5}
		C	8.2×10^{-5}	1.3×10^{-4}
		D	1.2×10^{-4}	1.2×10^{-4}
		E	2.4×10^{-5}	7.3×10^{-5}
pH4	Dead	A	0.37	2.0×10^{-4}
		B	0.61	2.9×10^{-4}
		C	2.6	5.7×10^{-4}
		D	4.3	1.9×10^{-3}
		E	0.86	5.7×10^{-4}

n.d. = not detectable

Upon examination of the Ni uptake data from Trials 1 and 2, it becomes obvious that to obtain maximum Ni uptake, isolate 8 should simply be grown at pH 4 and killed using ethylene-oxide gas sterilization prior to exposure to a pH 4 metals solution (Table 4, treatments "e" and "A", and Figure 3). The Fe uptake should be minimized with this procedure (Table 3, treatments "e" and "A", and Figure 3) and Co should not be uptaken (Table 5). Copper uptake will be less than could be obtained (Table 6, Figure 3), however, pH manipulation of the metals solutions would obviously be incompatible with maximum Ni uptake (Table 4) and minimum Fe uptake (Table 3).

Very high uptake values were reported for several of the metals in Tables 3 to 6. These values may be slightly over estimated because the biofilms formed on the glass beads at both pH 4 and pH 7 were very thin and our dried biomass values were therefore negligible. However, because we had viable cell count data per unit surface area (Table 7), we were able to estimate the dried biomass present by using the published dry weight of an Escherichia coli cell (2.8×10^{-13} g). Unfortunately, we were not able to correct for the biomass of the polymer itself, which will result in an over estimation.

Table 7

Dried Weight Estimations for the Biofilm Biomass Grown on Glass Beads for the Metal Uptake Trials

Isolate	# cells/ 5 beads	Dry weight/ 5 beads (g)	Dry weight (g/cm^2)
F8-pH7	3.0×10^{6}	8.4×10^{-7}	5.94×10^{-7}
F8-pH4	1.0×10^{2}	2.8×10^{-11}	1.98×10^{-11}
F2-pH7	2.0×10^{6}	5.6×10^{-7}	3.96×10^{-7}
F2-pH4	3.0×10^{5}	8.4×10^{-8}	5.94×10^{-8}

Note: surface area of 5 beads = 1.414 cm^2

This work constitutes discrete progress towards the expanded Objective #4 because this bacterial biofilm material can readily and very economically be produced and added to the effluent system to trap and recover specific metals both for their inherent value

and to protect the environment. We are confident that further manipulation (pH shifts, growth pH, nutrient shifts) will produce biomaterials of even greater avidity and specificity for the recovery of metals from mining effluents.

CONCLUSIONS

1. Bacteria exist within the Falconbridge effluent system that are capable of producing biofilms with a well-developed ability to trap nickel and copper.

2. Dead biofilm material appears to be preferable for metal binding operations because of its reduced ability to bind Fe.

3. Species selection has already provided bacterial strains capable of trapping Ni without binding significant quantities of Fe.

4. Dead biofilms may be more avid and more specific in metal trapping than are live biofilms produced by the same organism. For example, Co is more readily uptaken by dead F2 biofilms than by live F2 biofilms.

5. pH manipulation of the metals solution to which the biofilms are exposed can alter the avidity of metal binding. Copper uptake can be enhanced significantly using this protocol.

Figure 1: Trial 2 treatments followed for F2 and F8 isolates grown at pH 7.0

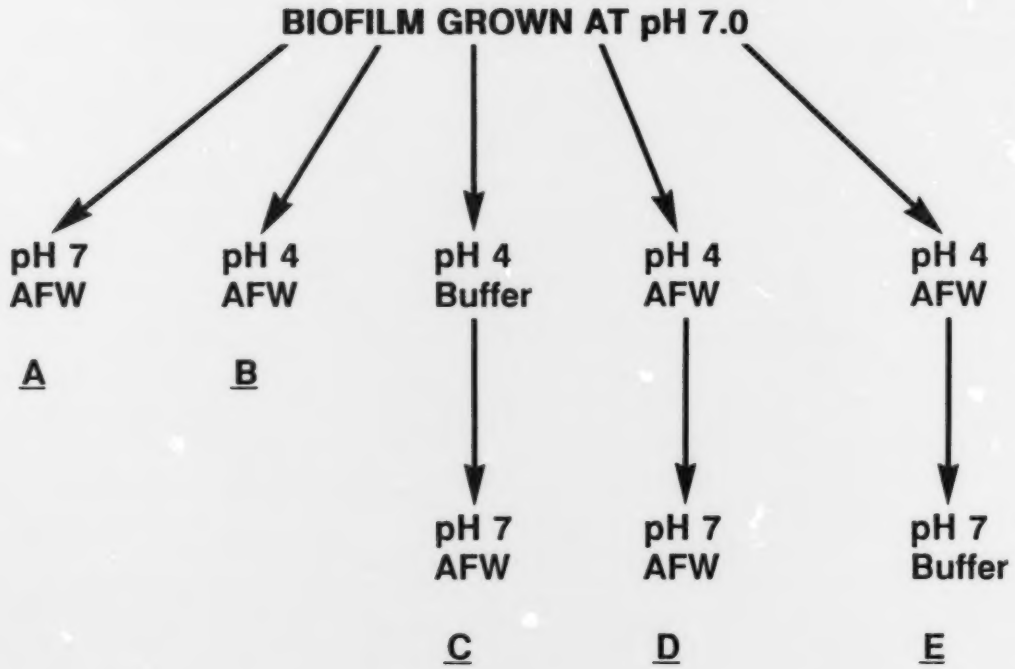

Figure 2: Trial 2 treatments followed for F2 and F8 isolates grown at pH 4.0

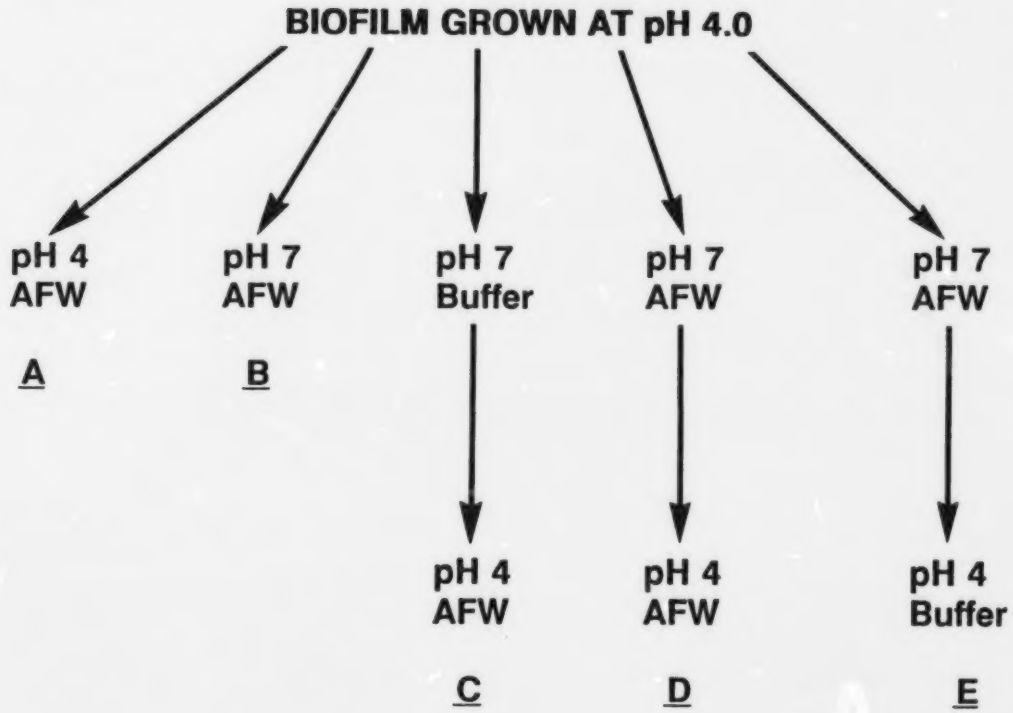

Figure 3: Nickel, iron and copper uptake by a dead F2 biofilm which had been grown at pH4. The treatments used are as in Figure 2